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Restoration of plant diversity in permanent grassland by seeding: Assessing the limiting factors along land-use gradients

Martin Freitag[1] | Valentin H. Klaus[2] | Ralph Bolliger[3] | Ute Hamer[1] | Till Kleinebecker[4] | Daniel Prati[3] | Deborah Schäfer[5] | Norbert Hölzel[1]

[1]Institute of Landscape Ecology, University of Münster, Münster, Germany

[2]Institute of Agricultural Sciences, ETH Zürich, Zürich, Switzerland

[3]Institute of Plant Sciences, University of Bern, Bern, Switzerland

[4]Department of Landscape Ecology and Resource Management, Justus Liebig University Gießen, Gießen, Germany

[5]Botanical Garden of the University of Bern, Bern, Switzerland

Correspondence
Martin Freitag
Email: martin.freitag@uni-muenster.de

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Abstract

1. Land-use intensification has caused dramatic declines in grassland biodiversity, and in Europe this decline has not yet been halted. Therefore, a major challenge in ecological restoration is to effectively restore plant diversity in permanent grasslands, that is, long-term managed grassland not being used as cropland. As species richness is often limited by seed dispersal, introducing locally absent species is crucial. Seeding diverse seed mixtures is an appropriate tool, but restoration outcomes are highly variable. Considering constraints by land-use intensity and plant functional traits during restoration could improve the efficacy of restoration efforts.
2. We established a full-factorial experiment with high-diversity seeding and topsoil disturbance in 73 grasslands along land-use intensity and productivity gradients and evaluated the restoration success over 5 years. In addition, after 5 years we assessed the number of established species and tested whether specific traits promoted the establishment of sown species in interaction with productivity.
3. Plant diversity increased considerably when seeding and disturbance were combined while seeding alone only slightly increased species richness. Along land-use gradients, we found no direct effects of grazing or mowing on the establishment of sown species, but fertilization indirectly limited establishment in the combined treatment via a negative effect of productivity.
4. Functional traits structured the establishment of sown species, especially in the combined treatments, but effects varied with productivity. Species with low seed mass and low specific leaf area had greater establishment success in low-productivity grasslands, whereas species with high seed mass had greater establishment in high-productivity grasslands.
5. *Synthesis and applications*. Our results highlight the potential of using diverse seed mixtures for biodiversity restoration in a wide range of permanent grasslands, especially when combined with topsoil disturbance. However, productivity both limited restoration success and affected establishment via an interaction with plant functional traits. As productivity is a major constraint in grassland restoration,

we strongly recommend to first stop fertilization and reduce soil nutrient loads in highly productive grasslands. Restoration by seeding, with or without disturbance, should only be applied when productivity is reduced and the local environment should be considered to compile site-specific seed mixtures to maximize restoration success.

KEYWORDS

dispersal limitation, fertilization, functional traits, land-use intensity, productivity, seed addition, seedling establishment, trait–environment interactions

1 | INTRODUCTION

Land-use intensification has dramatically impacted both biodiversity and ecosystem service provision of grasslands (IPBES, 2019; Newbold et al., 2016). Europe, for example, faces alarming and ongoing declines in area and deteriorations in conservation status of permanent grassland (Janssen et al., 2016), that is, continuously managed grassland that was not used as cropland for at least 5 years. Severe declines in plant species richness have been especially caused by intensive fertilization (Gross et al., 2009; Socher et al., 2012) such that even common plant species have decreased in abundance at alarming rates (Jansen et al., 2019). Grasslands therefore are a major target of ecological restoration (Blakesley & Buckley, 2016). Yet, our predictions of restoration success are often inaccurate because outcomes vary greatly among restoration efforts, mostly because of widespread variability in restoration approaches and site conditions (Brudvig et al., 2017).

To restore the local plant diversity in grasslands, seed dispersal limitation must be overcome (Clark et al., 2007; Münzbergová & Herben, 2005; Myers & Harms, 2009). Most grassland species are short-distance seed dispersers (Coulson et al., 2001; Sperry et al., 2019) and do not form persistent soil seed banks (Kiss et al., 2018). Therefore, a mandatory step is to actively introduce locally absent species. Seed sowing is a commonly applied technique that enables one to tailor the species composition to a target community (Kiehl et al., 2010). In addition, diverse seeding could be implemented in agricultural routines to increase diversity at larger scales. Restoration by seeding has been successfully applied on ex-arable land (Pywell et al., 2002; Wagner et al., 2020) and in low- and high-productivity grasslands (Ladouceur et al., 2020; Pywell et al., 2007; Zobel et al., 2000). Yet, most studies considered single or few sites, which makes it difficult to assess the significance of key constraining factors across real-world gradients of land-use intensity and productivity.

In the germination and seedling stages of a plant, safe microsites such as gaps within the established vegetation are crucial (Grubb, 1977). These gaps in the established sward are often created via topsoil disturbance, which enables seed–soil contact and amplifies seed germination through reduced competition and increased light availability at the soil surface (Bischoff et al., 2018; Myers & Harms, 2009). Such microsites create a safe regeneration space even in productive grasslands (Foster et al., 2004), but competition with the regrowing vegetation sward in the years after disturbance may impede long-term establishment in productive sites (Dickson & Foster, 2008). This competition for light can be reduced with repeated biomass removal through mowing or grazing, thereby increasing the establishment success (Dickson & Foster, 2008). Additionally, grazing creates microsites by trampling, which could further facilitate establishment (Kladivová & Münzbergová, 2016). Although high-diversity seed mixtures increased diversity even in the long run (e.g. Maccherini & Santi, 2012), it remains largely unclear how topsoil disturbance interacts with grazing or mowing to shape establishment in managed grasslands. Determining how topsoil disturbance, grazing and mowing affect the long-term establishment of seeds along productivity gradients can improve outcome predictions and may provide guidance on how to prioritize sites for restoration actions.

To improve restoration success and maximize ecosystem functions, plant functional traits can help to compile targeted seed mixtures (Laughlin, 2014). Because traits, especially canopy height, specific leaf area and seed mass, represent fundamental trade-offs of plant strategies in response to the environment and affect competitive and reproductive abilities (Westoby, 1998), they can be used, for example, to select potentially successful species to increase restoration success (Pywell et al., 2003). Furthermore, a deeper understanding of assembly mechanisms in restoration enables us to restore communities targeted to specific environmental conditions (Laughlin, 2014; Török & Helm, 2017). Assembly mechanisms such as environmental filtering influence restoration success because it structures the establishment of species with particular traits (Balazs et al., 2020; Zirbel & Brudvig, 2020). Knowledge on how traits interact with environmental gradients, such as productivity and land-use intensity, can assist in the selection of species based on local site conditions.

Here, we aimed to assess the long-term restoration success using a diverse and regionally adapted seed mixture along gradients of land-use intensity and productivity, with and without prior topsoil disturbance. We established a full-factorial experiment in 73 grasslands ranging from low-productivity sheep pastures and single-cut meadows to highly fertilized meadows and mown pastures with four cuts pear year or intensive grazing. Short-term results from this experiment showed that intensively used, species-poor

grasslands increased strongly in species richness when seeding and topsoil disturbance were combined while seeding alone had no effect on diversity (Klaus et al., 2017). Here, we assessed how land use, productivity and plant functional traits structure the long-term establishment of sown species. Specifically, we investigate (a) the effectiveness of seeding, topsoil disturbance and their combination to enhance plant diversity over time, (b) the degree to which land-use intensity and productivity constrain the establishment of sown species and (c) how traits, trait–environment and trait–management interactions affect the establishment of sown species during restoration.

2 | MATERIALS AND METHODS

2.1 | Study design

We analysed 5 years of a seeding and topsoil disturbance experiment in 73 grasslands as part of the Biodiversity Exploratories project (Fischer et al., 2010). We installed the experiment in three German regions that span gradients in soil characteristics, elevation and climate that are representative of large parts of Central Europe. The regions comprise (a) the calcareous mid-mountain range Biosphere Reserve Schwäbische Alb (48.4°N, 9.4°E), (b) the calcareous low-mountain range National Park Hainich-Dün and surroundings (51.1°N, 10.4°E) and (c) the postglacial landscape of Biosphere Reserve Schorfheide-Chorin (53.0°N, 14.0°E). While grasslands in the Schwäbische Alb and Hainich-Dün are restricted to mineral soils, grasslands in Schorfheide-Chorin are situated on drained fen soils or slightly acidic sandy soils (Fischer et al., 2010). Within each region, we selected 23–25 permanent grasslands along gradients of land-use intensity, productivity and corresponding plant diversity, ranging from unfertilized sheep pastures with 70 species per 16 m^2 to highly fertilized, productive and frequently mown grasslands with 10 species per 16 m^2 (Socher et al., 2012). To quantify land-use intensity, farmers were interviewed each year to inquire on the amount of fertilizer used (in kg N/ha), mowing frequency and grazing intensity (livestock unit grazing days $\times$ ha^{-1}; Vogt et al., 2019). Here, we used averaged fertilization, mowing and grazing intensities from 2014 to 2018 accessed via the LUI calculation tool (Ostrowski et al., 2020) implemented in BExIS (http://doi.org/10.17616/R32P9Q).

We established a 2 $\times$ 2 factorial experiment with seeding and topsoil disturbance in 73 grasslands. This resulted in four 7 m $\times$ 7 m treatments (control, seeding only, seeding and disturbance, and disturbance only), which we arranged in a rectangle with 2 m distance between treatments (see Appendix S1). In October 2014, we applied the disturbance treatment by mechanical perturbation of the established sward and the topsoil down to 10 cm with a rotary harrow or a rotary cultivator. The topsoil disturbance created a high proportion of bare ground (~50% in the following spring; Schäfer et al., 2019) and was similar to common agricultural re-seeding practices. We did not remove root and shoot fragments of the disturbed sward so that plants could possibly regrow. See Klaus et al. (2017) for further details on the experimental design.

For the seeding treatments, we used commercially available regional seed mixtures from certified seed producers, composed of genotypes that are assumed to be adapted to regional environmental conditions (Bucharova et al., 2017). The mixtures consisted of common and less-common species, selected from the species pools of each individual region (Fischer et al., 2010) and included grasses, legumes and forbs. Seeding density per species depended inversely on seed mass (high: 100 seeds/m^2 if seed mass <1 mg, medium: 34 seeds/m^2 between 1 mg and 10 mg, low: 17 seeds/m^2 >10 mg). Two-thirds of the mixture were sown in November 2014 and one-third in March 2015. In total, we sowed 5.37 g/m^2 and 66 species in Schwäbische Alb, 4.11 g/m^2 and 52 species in Hainich-Dün and 3.47 g/m^2 and 47 species in Schorfheide-Chorin (no regional seeds of legumes available from seed producers for this region). To estimate germination rates, we sowed 60 seeds per species and region in standard potting soil. We placed the pots outdoors from December 2015 until March 2014 in Bern, Switzerland, to allow for cold stratification, relocated the pots thereafter to a greenhouse and counted the emerging seedlings over 3 months.

We surveyed vegetation and measured productivity from 2015 to 2019 annually in May before the first mowing or grazing took place. On all four 7 m $\times$ 7 m treatments in each of the 73 grasslands, we recorded all vascular plant species on 2 m $\times$ 2 m quadrats and estimated percentage cover. As a measure of competition, annually we clipped all living plant biomass on one square meter on the control treatments (four 0.25 m^2 squares, different but close-by locations each year, see Appendix S1). We dried the biomass for 48 hr at 80°C and weighed it to the nearest gram. In 2018, we could not access three grasslands due to early land use (i.e. missing data for 12 observations). In total, we recorded 1,448 observations over the 5 years.

2.2 | Analysis

2.2.1 | Seeding and topsoil disturbance effects on plant diversity

We analysed how seeding and topsoil disturbance influenced plant diversity over time. We used two complementary diversity indices, namely species richness and effective number of species S_{PIE}, which accounts for community evenness by weighing abundant species more than rare species. We calculated evenness as the probability of interspecific encounter $\mathrm{PIE} = \sum_{i=1}^{S} p_i^2$, where S is the species richness and p_i is the relative cover of species i, and transformed PIE into an effective number of species $S_{PIE} = 1/(1 - \mathrm{PIE})$ (Chase et al., 2018; Jost, 2006). S_{PIE} equals species richness when all species have equal abundance, but S_{PIE} decreases when cover is more unevenly distributed across species. We modelled species richness and S_{PIE} as a function of seeding, disturbance and year, including all their interactions, and assumed

Poisson error structure for species richness and Gamma error structure for S_{PIE}, both with log-link. We treated year as categorical because effects over time were nonlinear. We included varying intercepts for grassland site to account for the experimental design and varying intercepts for year within region (5 years times three regions) because weather conditions and observers varied between years and regions. The multi-level models were estimated in a hierarchical Bayesian framework using the Stan probabilistic language (Stan Development Team, 2020) accessed via the package BRMS v2.13.0 (Bürkner, 2017) in R v4.0.3 (R Core Team, 2020). For all models, we specified weakly informative normal priors with zero mean for fixed parameters and default vague priors for the intercepts (see Appendix S2). We ran four parallel chains and 10,000 iterations (5,000 discarded as burn-in) and ensured convergence with R-hat values being <1.01 for all parameters. Model fit was assessed with posterior-predictive checks using the package BAYESPLOT v1.7.2 (Gabry et al., 2019; Gabry & Mahr, 2019; see Appendix Figure S2). We report 90% credible intervals (CrI) for posterior samples, which do not overlap zero when 95% of the posterior is either above or below zero, and we calculated conditional and marginal Bayes R^2 (Gelman et al., 2019) to assess how much variation is explained by the treatments or varying intercepts.

2.2.2 | Direct and indirect effects of land use on establishment

To evaluate how grazing, mowing and productivity modify or create regeneration spaces and hence determine the number of established seeding species, we modelled the absolute difference in sown species richness between the seeding treatments and control (Δrichness) as well the difference in total species richness. We fitted two separate multivariate models for seeding (only) and seeding and topsoil disturbance (combined) treatments in the first year and in the fifth year. Multivariate models sample the parameters jointly for the sub-models and, thus, allow modelling response variables measured on the same experimental unit. We expected direct effects of grazing (log-transformed grazing days/ha), mowing frequency and productivity (log-transformed g/m^2) on establishment and included region effects as well as an effect of Δrichness in year 1 on Δrichness in year 5 (see also Figure 2). Because fertilization intensity (log-transformed kg Nitrogen/ha) was closely correlated to mowing frequency ($\rho = 0.66$, Vogt et al., 2019), we included only an indirect effect of fertilization on Δrichness via productivity. We modelled productivity with Gamma distribution and log-link and assumed Gaussian errors for Δrichness. The repeated biomass measurements were simultaneously modelled as arising from a normal distribution with a mean equal to the 'true' latent productivity of each grassland site and measurement error σ. We chose arithmetic means and standard deviations of harvested biomass as informed priors for the means of latent productivity (i.e. varying between grasslands), and a weakly informative prior for the measurement error σ (see Appendix S4). The multivariate models were estimated using RSTAN v2.19.3 (Stan Development Team, 2020).

2.2.3 | Plant functional traits effects on establishment

To explore how functional traits affect the establishment of sown species 5 years after seeding along the productivity gradient, we modelled establishment with logistic regression. We assumed sown species to have successfully established in the seeding treatments if they were present in year 5. Because some of the sown species may have been present before the start of the experiment, we only considered a species as successfully established when the species was absent on either the control or disturbance-only treatment in any year. We kept 2,746 observations, because sown species were already present in the respective grasslands in 37% of the cases in the Alb region, 36% in the Hainich region and 21% in the Schorfheide region (see Tables S3 and S4). This definition is conservative, especially for common species, but reduces the number of false-positive observations where a sown species was already present. From the LEDA Traitbase (Kleyer et al., 2008), we extracted specific leaf area (relative growth rate), canopy height (competitive ability of the adult plant) and seed mass (energy for seedling recruitment) and averaged the trait values by species.

To test for the effects of traits and trait–environment interactions on establishment, we modelled binary establishment with logit-link function as a function of height (log.), seed mass (log.) and specific leaf area. We included estimated live seeding density (seeding density multiplied by germination rate) as a continuous predictor. Informed by a negative effect of productivity on the number of established species, we initially included productivity (log-transformed, as a measure of competition) and interactions of traits with productivity. We included varying intercepts for both species identity and grassland site due to the multi-level structure and scaled all predictors to unit standard deviation. Productivity was again modelled as latent (see Appendix S5).

To test for trait–grazing and trait–mowing effects, we fitted three additional models with (a) trait–grazing interactions, (b) trait–mowing interactions and (c) both trait–grazing and trait–mowing interactions in addition to trait–productivity interactions. To judge whether including grazing and mowing interactions improved the prediction of establishment, we compared models based on the expected log point-wise predictive density from leave-one-out cross-validation (Vehtari et al., 2017, 2020).

3 | RESULTS

3.1 | Seeding and topsoil disturbance effects on plant diversity

High-diversity seeding had clear positive effects on species richness over the 5 years of this study, but less so on S_{PIE}, which accounts for community evenness. The seeding effect on species richness increased steadily over time, from slightly positive in the first year to clearly positive in the fifth year (seeding $\beta = 0.04$, 90% CrI: −0.0 to 0.1, plus seeding*5th year $\beta = 0.09$, 90% CrI: 0.0 to 0.2; Figure 1a;

Appendix Table S1). Transformed to the response scale with average species richness of 25.7 per 4 m^2 on the control, this resulted in an increase in 3.1 species in year 5 (Figure 1c). Seeding alone had no clear effect on S_{PIE} (Figure 1a,f), which suggests that increases in richness were compensated by decreases in evenness because the sown species established with low abundance. Topsoil disturbance led to temporary increases in both species richness and S_{PIE} (Figure 1a,b,e), indicating the germination of ruderal species and an increase in evenness after disturbance.

Both species richness and S_{PIE} considerably increased when seeding and topsoil disturbance were combined. For species richness, the seeding and disturbance interaction was clearly positive in the first year but became weaker over time (seeding*disturbance $\beta = 0.25$, 90% CrI: 0.2 to 0.3, plus seeding*disturbance*5th year $\beta = -0.16$, 90% CrI: −0.3 to −0.1; Figure 1a,d; Appendix Table S1). S_{PIE} was also positively affected by the seeding*disturbance interaction (seeding*disturbance $\beta = 0.15$, 90% CrI: 0.0 to 0.3), but temporary increases in S_{PIE} were mainly driven by the disturbance effect (e.g. disturbance*2nd year $\beta = 0.15$, 90% CrI: 0.0 to 0.3; Figure 1a,e). On the response scale, the combination of seeding and disturbance led to an increase in species richness by 8.3 species in the fifth year (Figure 1d) and an increase in S_{PIE} by 0.6 (Figure 1g), given an average S_{PIE} of 6.2 on the control.

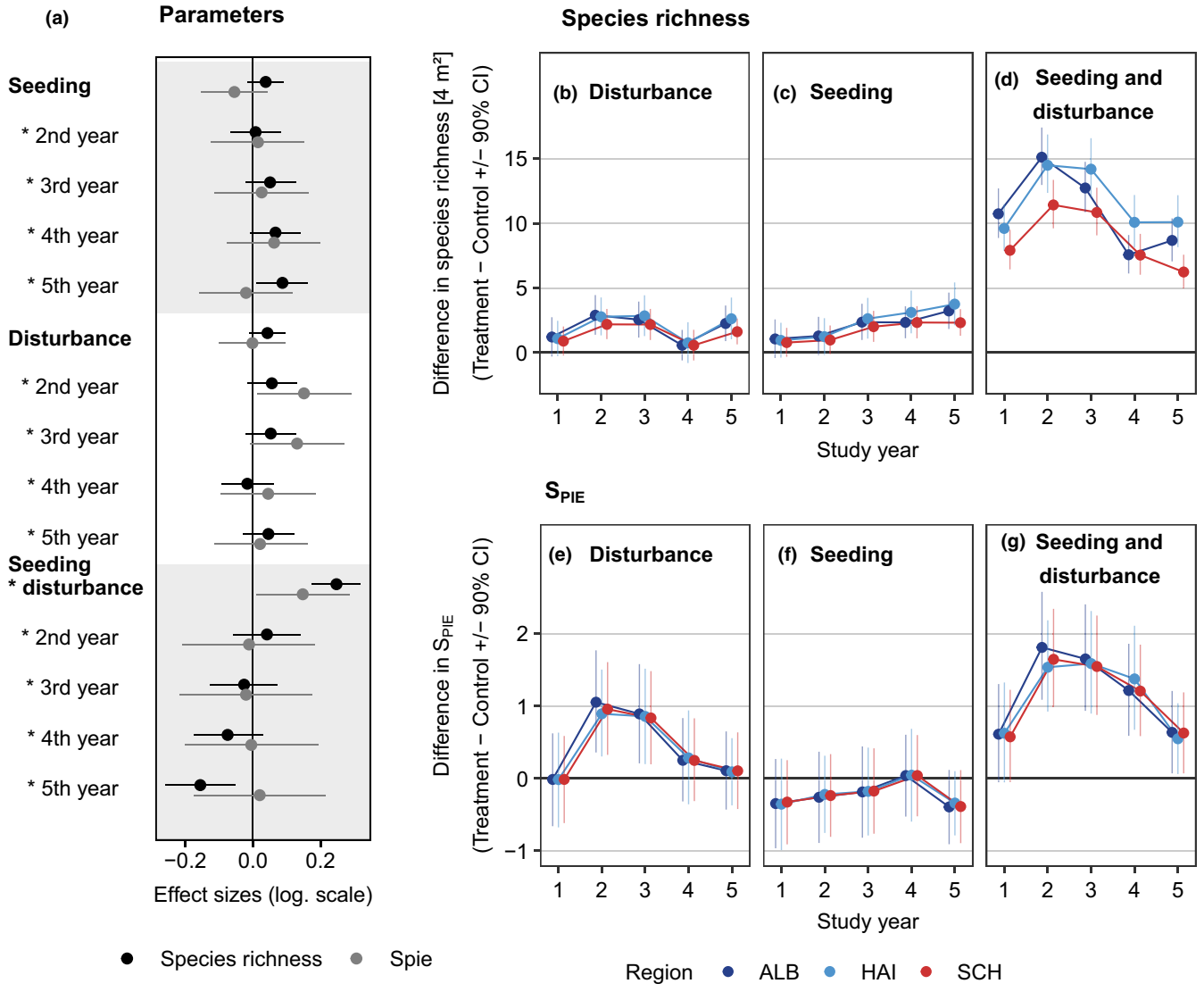

FIGURE 1 Effects of seeding and topsoil disturbance on species richness and S_{PIE} over time. (a) Effect sizes of seeding, disturbance and the seeding and disturbance interaction, as the main and interacting effects with years, for species richness and S_{PIE} (intercept, year main effects and varying intercepts not shown, see Appendix Table S1). Both diversity measures were modelled with log-link assuming Poisson and Gamma error structure, respectively. Panels (b–g) display differences between treatments and the control on the response scale. Seeding alone had small positive effects on species richness, which increased over time (c) while this was not found for S_{PIE} (f). The combination of seeding and disturbance had a clear positive effect on both diversity metrics, although it was weaker for S_{PIE}. To calculate differences between treatments and control, we predicted species richness and S_{PIE} based on additive parameter effects (a), transformed predicted values to the response scale and calculated the difference in predicted values between treatments and control. Points indicate medians along with 90% CrI

3.2 | Direct and indirect effects of land use on establishment

We found no direct effects of grazing or mowing on the number of established species (Δrichness), but fertilization indirectly limited the long-term establishment on the combined treatment via a negative effect of productivity on Δrichness in year 5 (Figures 2b,d and 3; Appendix Tables S2 and S3). Δrichness in the first year was less related to productivity or land use, underlined by lower R^2 values for both treatments compared to the fifth year (Figure 2). Instead, Δrichness of the combined treatment in the first year was higher when considering all species (Intercept α = 9.84, 90% CrI: 8.4 to 11.2; Figure 2b) compared to Δrichness of seeding species (Intercept α = 6.10, 90% CrI: 5.2 to 7.0; Figure 2d), which suggests a considerable

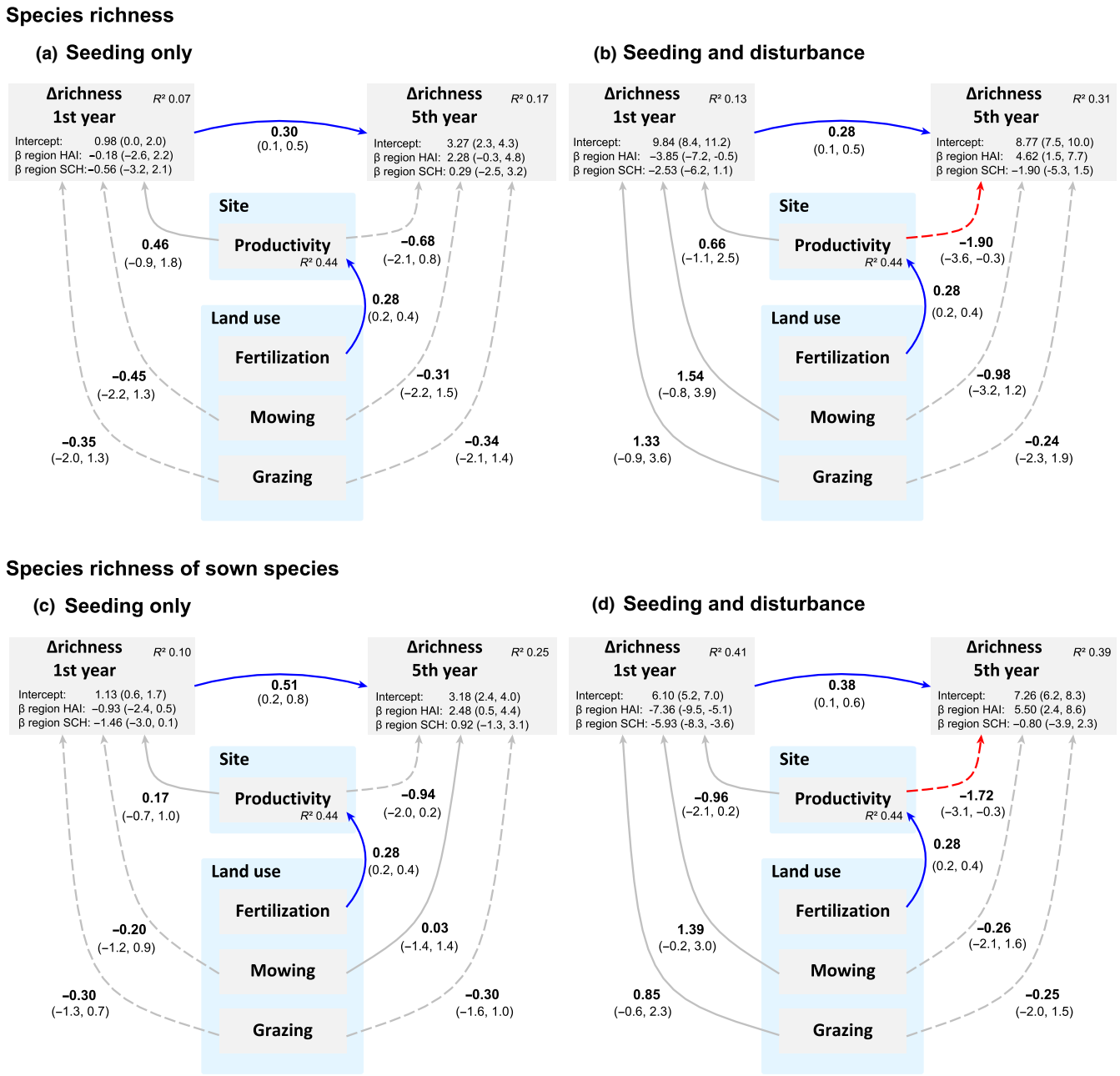

FIGURE 2 Direct and indirect land-use effects on the number of established species (Δrichness, the difference in species richness between treatment and control) for total species richness and sown species richness. Land-use effects on Δrichness were absent on the seeding-only treatment (a, c). Fertilization had an indirect negative effect via productivity on Δrichness in the fifth year for both (b) total and (d) sown species richness on the seeding and disturbance treatment. In the first year after disturbance, the larger Δrichness for total species richness indicates a considerable increase in unsown species on the seeding and disturbance treatment, which almost completely vanished in the fifth year. Positive paths are shown as solid and negative paths as dashed lines along with standardized regression coefficients and 90% CrI, in blue (positive) and red (negative) when the CrI does not overlap zero. Fixed effects of region are displayed in the Δrichness boxes

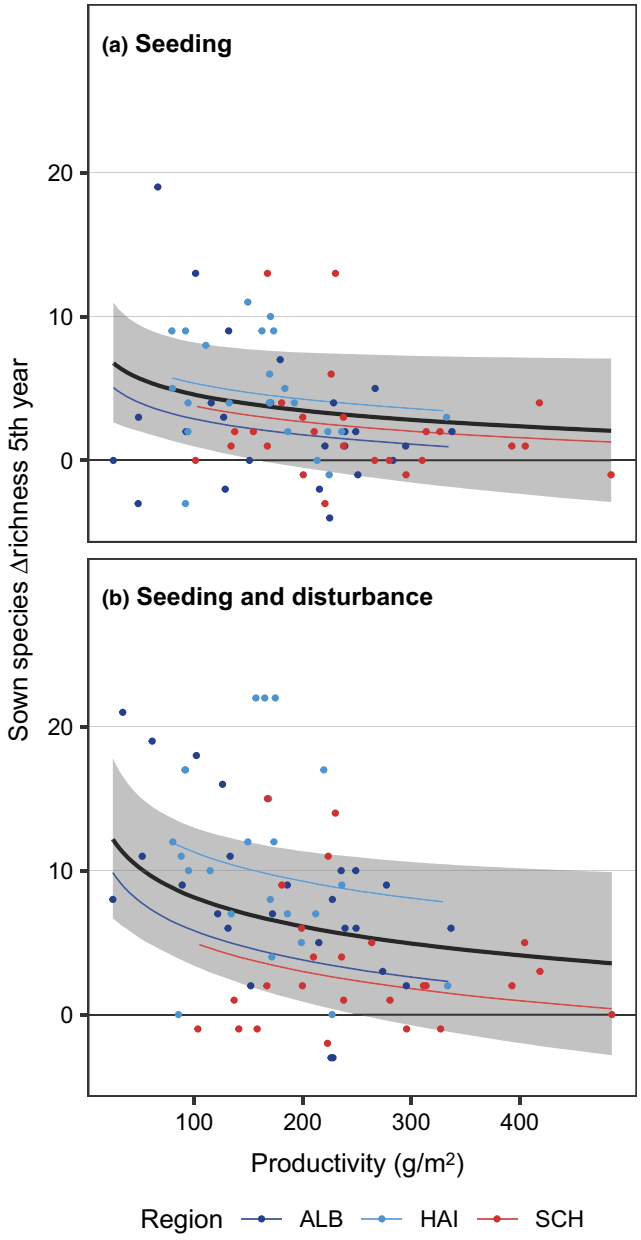

FIGURE 3 Conditional effect of productivity on the number of established sown species as compared to the control (Δrichness) in year 5. (a) Overall sown species Δrichness was lower and the effect of productivity weak on the seeding-only treatment, (b) but sown species Δrichness declined with productivity on the combined treatment. Lines denote the median and 90% CrI of sown species Δrichness across all 73 grasslands (grey) and within regions (coloured), conditioned on mean values of the other predictors (see Figure 2)

but temporary contribution of ruderal species to Δrichness after topsoil disturbance. However, sown species Δrichness increased until the fifth year, and the difference to Δrichness considering all species decreased considerably (Figure 2b,d). The negative effect of productivity on Δrichness was less clear for the seeding-only treatment but turned from neutral to weakly negative over time for sown species Δrichness (Figure 2a,c).

3.3 | Plant functional traits effects on establishment

Plant functional traits considerably influenced establishment, but effects of traits on establishment interacted with productivity. Establishment was consistently predicted by productivity, specific leaf area and their interaction (Figure 4c,d). Productivity decreased establishment drastically such that the probability of successful establishment in year 5 was almost zero in productive grasslands for the seeding-only and seeding and topsoil disturbance treatments (insets in Figure 4). Species with low specific leaf area had higher average establishment success, but trait–productivity interactions for both seeding treatments indicate that species with low specific leaf area established increasingly better at low-productivity sites (Figure 4c,f). The effects of height and seed mass were less clear (Figure 4a,b,d,e), but the trait–productivity interactions suggest that tall species with high seed mass established better at highly productive sites in the combined treatment (Figure 4d,e). We tested for additional trait–grazing and trait–mowing interaction effects on establishment but based on leave-one-out cross-validation, neither interaction improved the prediction of establishment success (Appendix Table S7).

Live seeding density (mean 7.5 viable seeds/m^2, Appendix Figure S6) increased establishment rates for both seeding-only (β = 0.68, 90% CrI: 0.4 to 0.9, Appendix Table S4) and seeding and topsoil disturbance treatments (β = 0.88, 90% CrI: 0.7 to 1.1). The effects on establishment on the seeding-only treatment were less clear, as overall parameter uncertainty was higher and average establishment success lower (Intercept α = −4.02, 90% CrI: −4.7 to −3.4) compared to the combined treatment (Intercept α = −2.57, 90% CrI: −3.1 to −2.1). The marginal Bayes R^2 was considerably lower than the conditional R^2 for both seeding treatments (seeding only: median R^2_{marg} = 0.20, R^2_{cond} = 0.47; seeding and disturbance: median R^2_{marg} = 0.29, R^2_{cond} = 0.46), which implies that grassland site and species identity accounted for a considerable share of variance not explained by traits.

4 | DISCUSSION

The ongoing biodiversity crisis reflects an urgent need to restore plant diversity in grasslands wherever possible but predicting restoration success is remarkably difficult (Brudvig et al., 2017), and restoration actions need to be carefully adapted to local site conditions (Laughlin, 2014; Török & Helm, 2017). Our results highlight the potential for using high-diversity seed mixtures to restore plant diversity across a wide range of permanent grasslands, especially when combined with topsoil disturbance. However, productivity limited the overall establishment success of sown species and shaped establishment via an interaction with plant functional traits. We demonstrate that productivity is a major constraint for the restoration of plant diversity in managed grasslands, but productivity can be used as an indicator to plan restoration actions and select suitable target species along productivity gradients.

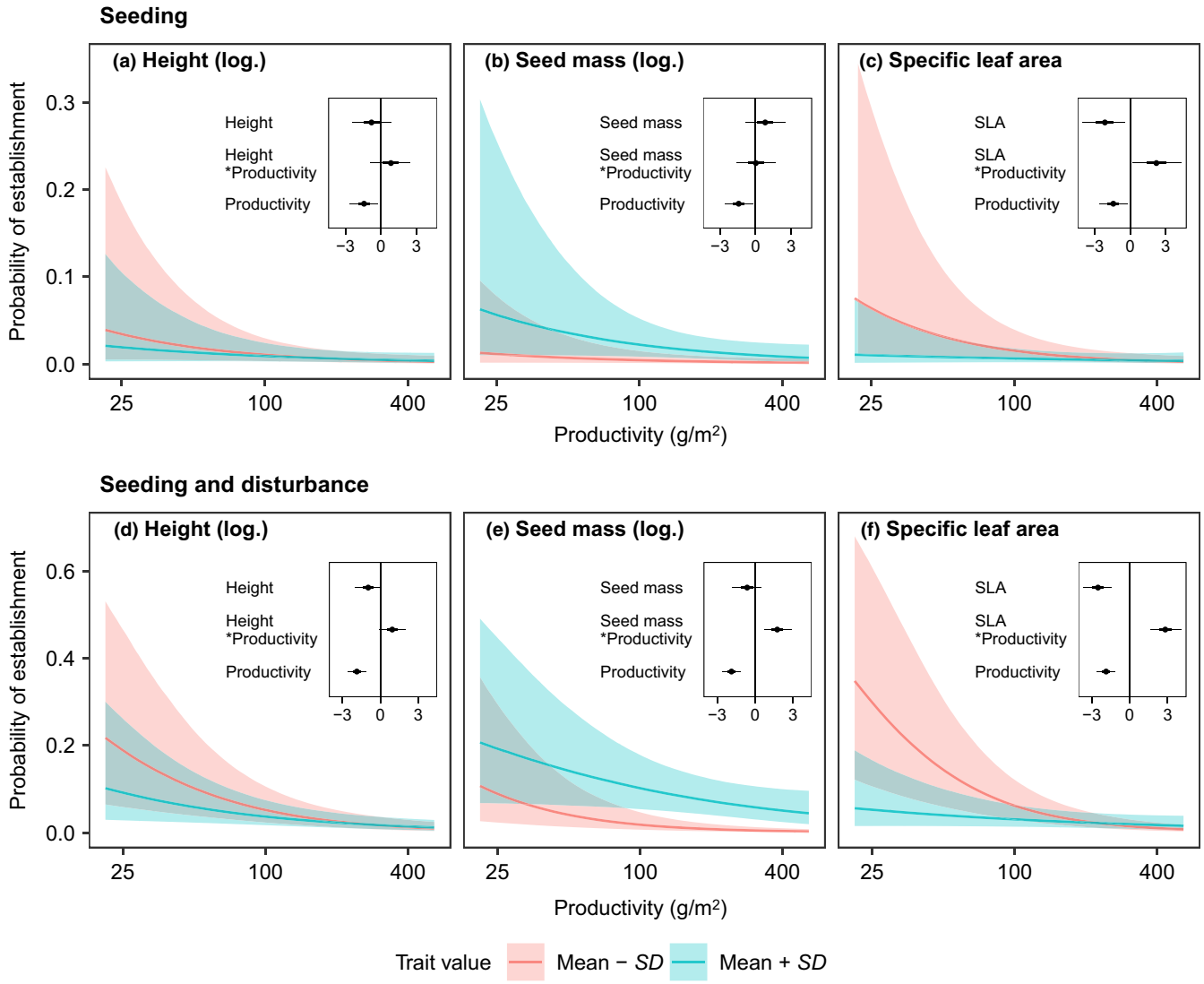

FIGURE 4 Conditional effects of plant height, seed mass, specific leaf area and productivity (log scale) on the establishment of sown species on (a–c) the seeding-only and (d–f) the seeding and topsoil disturbance treatments. Especially in the combined treatment, trait effects on establishment interacted considerably with productivity. Coloured lines denote the median and 90% CrI for low (mean minus one *SD*, red) and high (mean plus one *SD*, blue) trait values, conditioned on mean values of the other traits and mean live seeding density. In each panel, inset plots show the median and 90% CrI of the parameters used for prediction. See Table S4 for details

The most effective method for increasing plant diversity was the combination of seeding and topsoil disturbance. This finding is well in line with early results from this experiment (Klaus et al., 2017) and confirms previous studies, which conclude that species richness is largely limited by dispersal limitation and the lack of a persistent soil seed bank for most species (Clark et al., 2007; Klaus et al., 2018; Myers & Harms, 2009). Concurrent with previous research, disturbing the topsoil reduced microsite limitations and effectively created regeneration space for the seeded species to establish (Bischoff et al., 2018; Grubb, 1977; Münzbergová & Herben, 2005). Yet, over the 5 years of this study, species richness slowly increased in the seeding-only treatment as well. This suggests that at least some species can establish without topsoil disturbance, for instance when microsites are created by grazing (Kladivová & Münzbergová, 2016; Maccherini & Santi, 2012).

While we observed clear seeding effects on species richness, we found weaker effects on the effective number of species S_{PIE}, indicating that increases in richness were partly outweighed by decreases in community evenness due to low cover of the newly established species. In contrast to Ladouceur et al. (2020), who reported positive effects of seeding-only on S_{PIE} across a range of natural and semi-natural grasslands, in our study the lack of a seeding-only effect on S_{PIE} suggests that when topsoil disturbance was not applied, sown species had not yet become important members of the community. A possible explanation is that a higher average productivity of our grasslands limits establishment more strongly compared to the partially degraded grasslands included in the study by Ladouceur et al. (2020). The weaker positive effects of the seeding treatments on S_{PIE} due to increases in evenness suggest that creating microsites

is important for the introduced species to establish with noticeable cover, but it remains an open question as to how long these differences persist.

We found no direct effects of land-use intensity on the number of established seeding species, but fertilization indirectly limited long-term establishment via a negative effect of productivity. At first, the intensively used productive grasslands benefited most from seeding and topsoil disturbance (Klaus et al., 2017), but initial increases in species richness were recorded right after the set-up of the experiment, before the first mowing or grazing management, and were partly driven by ruderal species from the soil seed bank. While previous studies reported that productivity limits seeding successes in grasslands (Dickson & Foster, 2008; Foster, 2001), our study is the first to confirm this pattern along a realistic grassland productivity gradient. The increasing impact of productivity on establishment over time suggests that enhancing plant diversity through seeding is only an ephemeral success in productive grasslands and highlights that recruitment limitation is an at least as important constraint for species richness as dispersal limitation (Clark et al., 2007; Münzbergová & Herben, 2005). Concurrently, the number of established species correlated positively with the resident species richness (see Appendix S6), both of which are constrained by productivity (Socher et al., 2012). Thus, productivity limits both resident species richness and the establishment of introduced species, indicating that both species richness and the potential to introduce new species decrease with higher fertilization, which increases productivity.

Several studies have emphasized that soil fertility (and its determinant role for productivity) is one of the most important constraints to restoring species-rich grasslands (Dickson & Foster, 2008; Walker et al., 2004), and fertilization significantly contributes to this constraint. However, we were not able to identify a threshold of productivity at which restoration would change from success to fail. Our results suggest that the establishment success is generally low at productivity levels above ~250 g/m^2 above-ground green biomass in May, but the establishment probably still depends to a significant extent on further site characteristics. One such factor that determines seedling establishment might be litter biomass. While small amounts of litter can facilitate seedling recruitment in dry grasslands, dense litter (>500 g/m^2) largely inhibits recruitment (Loydi et al., 2013). However, we rarely observed significant amounts of litter in our grasslands, which is typical for regularly used Central European grasslands (Gross et al., 2009).

Interestingly, grazing and mowing had no effect on the number of established species. Grazing is assumed to create microsites by feeding and trampling and, hence, can increase the recruitment from seeds (Kladivová & Münzbergová, 2016), but effects of grazing on establishment are usually low (Maccherini & Santi, 2012; Rasran et al., 2007). In our study, bare soil cover was hardly related to grazing or mowing intensities (Appendix Figure S9). We suspect that productivity partly reflects the availability of microsites, because sparse vegetation with low above-ground biomass provides more space per se, or because the effects of grazing and mowing on microsites were overruled by productivity. Based on our results, we conclude that, in fertile grasslands, productivity must be reduced to ensure restoration success. The main focus in productive grasslands, therefore, must be to stop fertilization and reduce the soil nutrient loads, before fine-tuning the grazing and mowing management.

Plant functional traits clearly affected establishment. Sown species with a low specific leaf area consistently established better, independent of productivity. However, the patterns we observed for the seeding-only treatment were less clear, probably due to generally lower establishment rates and subsequently greater uncertainty in the relationships between traits and establishment. When seeding was combined with topsoil disturbance, we also found that low-competitive species with low canopy height and low seed mass (such as *Dianthus carthusianorum* or *Sanguisorba minor*) tended to establish better. In part, these main effects were possibly driven by the fact that the overall establishment rate decreased with productivity. At low levels of productivity, the plant community is characterized by species with a low specific leaf area (Allan et al., 2015), as conservative and slow-growing species with low specific leaf area have no disadvantage in establishing and are possibly better adapted to less fertile environments.

Such adaptions to the environment are reflected in the interactions between functional traits and productivity that shape establishment during restoration. For instance, in the combined treatment, species with high seed mass established better in productive sites. Having considerable energy stored in large seeds increases recruitment rates (Clark et al., 2007) and seedling survival rates (Metz et al., 2010), which seems to be a particular advantage in productive sites, where competition with the regrowing vegetation selects for the most competitive seedlings. In contrast, species with low specific leaf area established better at sites of low productivity. The fast resource-acquisition strategy of species with high specific leaf area is beneficial under nutrient-rich conditions, for instance resulting from intensive fertilization (Allan et al., 2015; Vries et al., 2012). Our results confirm the finding that this adaption to nutrient surplus in productive sites does not represent an advantage under conditions with less competition for light (Zirbel & Brudvig, 2020), where, for instance, water availability limits plant growth. We propose that sown species in grasslands are environmentally filtered by productivity and successful establishers possibly reflect the traits of the resident plant community (Ames et al., 2020; Breitschwerdt et al., 2015). Variation in establishment success along environmental gradients and trait–environment interactions are rarely studied, but insights on these relationships provide valuable information to prioritize restoration sites and compile site-specific seed mixtures to maximize success and ecosystem functions during restoration (Balazs et al., 2020; Laughlin, 2014).

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AUTHORS' CONTRIBUTIONS

D.P., U.H., N.H., V.H.K., T.K. and M.F. conceived the ideas and designed the experiment; V.H.K., D.S., R.B. and M.F. collected the data; M.F. analysed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data and code available via the Biodiversity Exploratories Information System https://doi.org/10.25829/bexis.30915-10 (Freitag et al., 2021). Code also available from https://github.com/martin-freitag/restoration-by-seeding.

ORCID

Martin Freitag https://orcid.org/0000-0002-3280-5941
Valentin H. Klaus https://orcid.org/0000-0002-7469-6800
Ralph Bolliger https://orcid.org/0000-0001-5383-9713
Ute Hamer https://orcid.org/0000-0002-3845-3983
Till Kleinebecker https://orcid.org/0000-0003-1121-2861
Deborah Schäfer https://orcid.org/0000-0001-9591-841X

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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